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Review Article

Biomarkers Used in Gall Bladder Cancer: A Review

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Abstract

Gall bladder carcinoma (GBC) is the most aggressive and common biliary tract malignancy. These arise from epithelial lining of gallbladder and intrahepatic and extra hepatic bile ducts. These are invasive type of adenocarcinomas. GBC represents 80 - 95% of biliary tract cancers worldwide. It is the fifth most common gastrointestinal cancer. Biomarkers play an important role in clinical trials, new therapies or adjuvant treatment modalities in oncology and also provide an estimate of prognosis. Biomarkers can be used in wide spread screening in order to detect asymptomatic patients in early stages of cancer. In this review we are discussing the epidemiology, risk factors and biomarkers used in detecting gallbladder cancer.

Keywords: *Cholelithiasis; Cholelithiasis; Biomarkers*

Introduction

Biomarkers are protein or protein fragment substances made by cancerous cells that can be easily detected in patient's blood or urine but not seen in healthy person [1,2]. If we go back to history, the first tumor marker was Bence Jone's protein for Multiple myeloma. In 1873 Multiple myeloma was described by Von Rustizky but Kahler who related the disease to Bence Jones proteinuria [3]. In 1965 Dr. Joseph Gold found a substance in a colon cancer patient's blood and feces and he called it as Carcinoembryonic antigen (CEA) [2].

In 1889, Stephen Paget put forward the "Seed and Soil" theory. According to this theory the basis of the present tumor microenvironment (TME) model of cancer metastasis. TME shows survival signals and pro-angiogenic factors, which are essential for tumor growth and metastasis. Biomarkers interplay between tumor cells and their microenvironment and lead to advances in early detection or prevention of metastasis [4].

The different phases of Gallbladder cancer includes metaplasia of normal epithelium or less hyperplasia, then dysplasia or intraepithelial neoplasia, carcinoma *in situ* and into invasive malignancy in approximately 15 years [5]. GBC patients shows high mortality rate because they often present in an advanced stage due underlying molecular processes often go up to decades unnoticed. Prognostic biomarkers and new potential markers would help to identify patients who might benefit from additional treatments.

Gall Bladder Carcinoma tends to increase as age advances. Average age was 67 years. Mortality increases with the age above 75 years [6]. Women are affected 2 to 6 times more than men. Co-expression of estrogen and progesterone receptors in increased in females than

males could the reason for female predominance in GBC [7]. Prevalence of GBC is high in Latin America, Asia (northern Indian females, Pakistani females and Korean males) eastern and central Europe [8]. Low prevalence seen in United States and most western and Mediterranean European countries. Worldwide, the mortality rate is higher in Mapuche Indians of Chile, Hispanics and North American Indians [9,10]. Mean survival rate for GBC patients 6 months, with a 5-year survival rate of 5%. The reason for high mortality rate is its proximity with liver and gallbladder lacks serosal layer and its perimuscular connective tissue is continuous with liver. So, its helps in easy invasion of liver malignancies and metastasis to gallbladder [10,11].

Risk factors

- **Cholelithiasis/gall stones:** 85% patients with gallbladder stones shows malignancy turnover. Large size stones more than 3 cms shows increased risks than smaller stones. Cholesterol gall stones also shows higher incidence. Stones creates local mucosal irritation and chronic inflammation leads to production of secondary bile acid, which is carcinogenic in nature [11,12].

- **Chronic inflammation:** Cholelithiasis causes frequent trauma leads to chronic inflammation that leads to deoxyribonucleic acid (DNA) damage. Inflammatory tissue proliferation leads to release of inflammatory cytokines, growth factors, nuclear factor kappa B, reactive oxygen and nitrogen species, prostaglandins and specific micro-ribonucleic acids (micro-RNAs). They act as mediators for DNA mutation, DNA methylation and angiogenesis [9]. Chronic inflammation leads to calcium deposition over the gallbladder wall this changes the appearance of gallbladder as "bluish" and brittle, hence it is called as "Porcelain gallbladder" [13].

- **Infections:** The organisms like *Salmonella* (e.g. *S. typhi* and *S. paratyphi*) and *Helicobacter* species, parasites like *Clonorchis* and *Opisthorchis*, causes GB cancer. Chronic bacterial cholangitis is the risk for biliary tract malignancy. Primary bile acids undergo bacterial hydrolysis may affect malignant transformation [14].

- **Environmental factors:** Nickel and cadmium metals, radon gas which is used in mine workers, methyldopa and isoniazid drugs, tobacco consumption habits are associated with GBC [11].

- **Systemic factors:** Obese people and those suffering from diabetes mellitus have an increased risk of developing gallbladder cancer [15].

- **Gallbladder polyps:** Solitary or sessile mass, large polyps, polyps associated gallstones are shows high rate of turning into malignancy.

- **Anatomy of pancreaticobiliary duct:** Congenital malformation in an anomalous junction of the pancreaticobiliary duct in which the pancreatic duct drains into the biliary tract outside the duodenal wall allowing pancreatic secretions to regurgitate into the bile ducts and gallbladder, thus leading to malignant changes in the mucosa [15].

- **Genetics:** A positive family history of gallbladder disease increases the risk of developing gallbladder cancer. Alterations of tumor suppressor genes [p53] or proto-oncogenes [K-ras], microsatellite instability and methylation of gene promoter areas leads to malignancy [16].

Important markers in GBC

Cyclin D1: Cell cycle and cell kinetics are important indicators of growth and behavior of various human tumors. In cell cycle the restriction point of G1-S is important. The cyclin D1, p16, Rb pathway plays as restriction factors in cell cycle. Elevated expression of cyclin D1

shortens G1 phase of the cell cycle and enhances the malignant transformation. A study conducted by Hui AM., *et al*. shows cyclin D1 overexpression was observed in adenoma and adenocarcinoma but not in normal epithelium of gallbladder. This study proved that cyclin D1 can be used as markers in early event in gallbladder carcinoma and also predicts poor prognosis [17-22].

E-cadherin: E-cadherin (CDH1) is a tumor suppressor gene located on chromosome 16q22.1. E-cadherin is critical for the development and maintenance of Ca^{2+}-dependent cell adhesion, polarity and differentiation of epithelial cells. According to one study showed loss of E-cadherin expression was seen 67% in Gall Bladder cancer, 94% in Chronic cholecystitis and 91% in Xantho Granulomatous Cholecystitis cases [23,24].

T-cadherin: T-cadherin also a member of cadherin family due to its tumor suppressor mechanism it act against carcinogenesis. Adachi., *et al*. conducted a study and suggested that T-cadherin decreases the expression of Akt3 and phosphorylated Akt molecules and hence prevents from malignancy [25].

Epidermal growth factor receptor (EGFR)

This is a protein which plays important role in local invasion and metastatic transformation. Lee and Pirdas in 1995, first to described EGFR correlation with bile duct tumors, gallbladder dysplasia, chronic calculous cholecystitis and gall bladder carcinoma.

Leone., *et al.* proposed that bile acids secretion in chronic cholestasis activates EGFR cascade activation which promotes cell survival, proliferation and inhibition of apoptosis [26,27].

Biomarkers in Gall Bladder Carcinoma		
1. ABCG2	16. CEA	31. MUC 1
2. Annexin A3	17. COX 2	32. MUC 2
3. ALDH	18. Cyclin D1	33. NANOG OCT-4
4. A L C A M gene	19. E-Cadherin	34. P-53
5. CA19-9	20. EGFR	35. Prosaposin
6. CA15-3	21. Eph B1	36. RAS
7. CA-125	22. Ephrin B	37. RAF
8. CA-242	23. ERBB 3	38. RCAS 1
9. CD -4	24. ERBB 4	39. SOX
10. CD-24	25. GLUT 1	40. SOX-2
11. CD-34	26. GLUT 3	41. SPOCK1
12. CD-44	27. H1F1 Alpha	42. T-Cadherin
13. CD-133	28. HER-2	43. TNF alpha
14. CD-147	29. Ki-67	44. Transgelin
15. CD-166	30. MEKERK ½	45. VEGF

HER-2

EGFR and HER2 both are tyrosine kinase receptors. They are proto oncogenes. Epidermal growth factor binds to EGFR or HER 2 receptors leads to angiogenesis and resistance to apoptosis. Hence over expression of these in tumor cells leads to poor prognosis [28]. Chaube., *et al.* study showed only 25%, Kawamoto., *et al*. reported 31.2%, Puhalla., *et al.* found 13% of the patients of GBC showed positive over expression of Her2/neu in specimens by IHC method [29]. A study conducted by Jeffrey., *et al*. observed HER2 amplification in 16% cases of biliary tract cancers [30].

KI-67

Ki-67 is a monoclonal antibody. It is expressed in all phases of the cell cycle except G0 and can be detected in the nucleus of proliferating tumour cells. Hence act as a proliferative marker [31]. Except for the resting phase G0, Ki67 antigen is expressed throughout during G1, S, G2 and M phases of cell cycle and important for cell division. Ki-67 levels are low in G1 and S phases and their peak level occurs in mitosis. According to Ohja., *et al.* 88.64% of Gall bladder carcinoma was positive for Ki-67 [32].

P53

P53 expression in GBC was first published by Kamel., *et al*. in 1993 [33]. It is located on the short arm of chromosome 17. It is a tumor suppressor gene and it inhibits cell proliferation by encoding 53-kd nuclear phosphorylation. Specific character of P53 is it undergoes point mutation. This mutation leads to loss of protein production and synthesis of mutated protein. Altered P53 protein or over expression of P53 found in GBC [34-36]. P53 mutations are seen in 40 - 70% of cases of GBC [37]. Roa., *et al.* observed in his study out of 191 cases, 45% of the sample expressed P53 over expression. Higher frequency of P53 expression is seen in advanced stage than in early stage [38]. P53 mutation seen in well differentiated and poorly differentiated adenocarcinoma, mucinous carcinoma and adenomatous squamous cell carcinoma [39].

Vascular endothelial growth factor (VEGF)

VEGF helps in neo-angiogenesis of tumors by nutrition supply and also have a metastatic potential. VEGF and VEGF receptors mediates endothelial cell proliferation. VEGF expression in gallbladder carcinoma helps in predicting post treatment clinical outcomes. VEGF-A is a potent proangiogenic agent. Increasing VEGF expression is related to progression of gallbladder carcinoma [5,40].

CD24

CD 24 is a small cell surface protein. It acts as a target for cancer treatment associated with monoclonal antibodies. According to study conducted by Song., *et al.* proposed that detection of NDRG2/CD24 co-expression helps in prediction of prognosis in Gall bladder carcinoma [41].

CD133 (Prominin-1 or AC133)

CD133, is a transmembrane protein. Tumorigenicity of CD13 cells shows it can act as a cell surface marker GBC. Chen., *et al.* study found that, down regulation of cd133 inhibits migration of gall bladder cancer cells by reducing Akt phosphorylation [42,43].

CD 44

It is a transmembrane glycoprotein involved in cell proliferation, angiogenesis, invasion and metastasis. Cd44v8-10 expression may be a prognostic marker of GBC. Shi., *et al.* identified CD44+CD133+ in primary GBC and hence act as a novel diagnostic and therapeutic target [44,45].

ALDH /Aldehyde dehydrogenases (ALDHs)

ALDH are a family of enzymes. ALDH1 and ALDH1A3 expression related to the clinical and pathologic features, carcinogenesis, progression and prognosis of gallbladder adenocarcinoma [46,47].

OCT-4

The Oct-4 gene, a POU- (Pit-Oct-Unc-) domain octamer-binding transcription factor usually expressed in human tumors but not in normal tissues. Oct-4 is an important marker in carcinogenesis and prognosis of gallbladder carcinoma [48].

CD147 (EMMPRIN)

It is a transmembrane protein of the immunoglobulin. Wu., *et al*. study findings suggested that CD147 and MMP-2 these two markers predict the prognosis of patients with gallbladder carcinomas [49].

CD34

CD34 expression is a useful biomarker in the gall bladder carcinoma helps diagnosis from cholelithiasis [50].

SOX

SOX (member of the SRY-related HMG-box). The SOX-4 is involved in tumorogenesis in many malignancies. Yadav., *et al.* study showed SOX -2, OCT-4, NANOG variants showed interactive role in treatment response of GBC [51,52].

CA 19-9

Carbohydrate antigen 19-9 and carcinoembryonic antigen (CEA) are the most commonly used markers in GBC. Studies have done using CA 19-9, CA19-9 combination with CA125, CA15-3, CA242 in patients with GBC [53].

p16

Ueki., *et al*. study evaluated on 68 GBC patients showed alterations in p16 gene [54]. High percentage of p16 gene involved in non-silent mutations, p16 methylation, and loss of chromosome 9p21–22 in GBC cases. Other studies like Quan., *et al*. and Ma., *et al*. also observed correlation of mutations in p53, p16 and Rb genes with the progression of GB [55,56].

Molecular biomarkers like RAS, RAF, MEKERK1/2, PI3k-AKT-mTOR and alterations in the MAP kinase pathway, FGF pathway, PI3K pathway helps in detecting GBC and biliary tract carcinoma [57,58]. In One study found that sample of 57 gallbladder cancer tumor somatic mutations in EGFR (4%), HER2 (10%), ERBB3 (12%), and ERBB4 (4%) [59]. According to Kalekou and Miliaras study reported high rates of EGFR alterations with overexpression of HER2 in GBC [60]. A study conducted on histological types including 221 cases of adenocarcinoma. EGFR, VEGF, HER2/Neu and p53 were assessed using immunohistochemistry. HER2/Neu, VEGF and EGFR are expressed in high proportion of gallbladder carcinoma [17].

During carcinogenesis accumulation of mutations of KRAS and loss of heterozygosity at 9p, 13q and 18q was observed. These genetic alterations changes tumor into invasive carcinoma [61,62]. Yamato., *et al.* conducted a study from Japan showed that expression of MUC1 and MUC2(Mucin core protein) at the protein and mRNA level in GBC patients. MUC 1 expression showed increased proliferation activity and malignant transformation and MUC 2 expression was related to lower proliferative activity in GBC [63]. Sergeant., *et al*. evaluated in 34 patients with gallbladder carcinoma where 50% of cases showed high expression of biomarkers like VEGF, HIF1alpha, GLUT1, GLUT3, CA9 and EGFR [64].

Conclusion

Expression of ABCG 2, EphB1, Ephrin B, Prosaposin and Transgelin are newer biomarkers that act as a resource for future studies of GBC. Autocrine TNF alpha is a tumor promoter gene and high expression SPOCK1 which is potential oncogene contributes the progression of GBC. Gallbladder Carcinoma MicroRNAs, cell-free nucleic acids (cfNA), mitochondrial DNA, autoantibody signatures and circulating tumor cells are new biomarkers would help GBC patients to early diagnosis and undergo further treatment option and for better prognosis.

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